The Distribution of the Desmosomal Protein, Plakophilin 1, in Human Skin and Skin Tumors

Ingrid Moll,* Hjalmar Kurzen,† Lutz Langbein,† and Werner W. Franke†

*Department of Dermatology, Mannheim Medical School, University of Heidelberg, Mannheim, and †Division of Cell Biology, German Cancer Research Center, Heidelberg, Germany

Desmosomes are predominant among the types of plaque-bearing adhering junctions found in human skin. These structures contain a set of desmosomal cadherins and cytoplasmic plaque proteins, the synthesis of which is differentiation dependent. As plakophilin 1, a member of the *armadillo* gene family, is an important accessory desmosomal plaque protein, we raised several monoclonal antibodies specific for this protein and applied immunohistochemical and immunoblotting procedures to study the distribution of plakophilin 1 in desmosomes in adult and fetal skin, psoriatic epidermis, various epithelial skin tumors, and keratinocyte sheets grown in culture. In epidermis, the spinous layers were prominently immunostained by plakophilin 1 antibodies, whereas the basal cell layer was only weakly stained and the stratum corneum was entirely unstained. The staining observed in psoriatic epidermis was somewhat heterogeneous. In hair follicles, the outer root sheath (ORS) was delineated in its suprabasal cell layers, with variable staining in its upper and lower parts. All basal cells of the ORS remained unstained, as did upper inner root sheath (IRS) and matrix cells of lower bulb. In eccrine sweat glands, the reaction was confined to inner dermal ductal cells, with the acini remaining unstained. The desmosomal immunostaining observed in basal cell carcinomas (BCCs) and squamous cell carcinomas (SCCs) was very heterogeneous: In general, junctions in well-differentiated stratified tumor regions were more intensely stained than sections of poorly differentiated and invasively growing BCCs and SCCs. Plakophilin 1 was also prominent in the desmosomes of keratinocyte sheets grown in culture. The cell type-specific, i.e., differentiation-dependent, distribution of desmosomal plakophilin 1 is discussed in relation both to the stratification of the cutaneous epithelia and to tumor differentiation and growth. ***Key words: desmosome/plakophilin 1/plakoglobin/desmoplakin/keratinocyte/epithelial tumor. J Invest Dermatol 108:139–146, 1997***

The cells of all types of epithelial tissues are connected to each other by various adhering junctions that typically exhibit cytoplasmic plaques beneath the plasma membrane (Farquhar and Palade, 1963; Luzi *et al*, 1987; Akiyama *et al*, 1995). Two types of adhering junctions occur in the skin:

1. The punctum adhaerens, a tiny plasma membrane segment containing classic cadherins such as E- and P-cadherin with a cytoplasmic plaque that contains plakoglobin as well as α- and β-catenin and several actin-binding proteins such as vinculin and α-actinin (e.g., Drenckhahn and Franz, 1986; Kemler, 1992, 1993; Tsukita *et al*, 1992).

2. The desmosome (macula adhaerens), with its typical disc shape, is the predominant junction structure of the various cutaneous epithelia (Allen and Potten, 1975; Kocher *et al*, 1981) and anchors intermediate-sized filaments of the cytokeratin (CK) type at the plasma membrane (for review see, e.g., Cowin *et al*, 1985). The desmosomal cadherins, i.e., the desmogleins (Dsg 1–3) and the desmocollins (Dsc 1–3; Mechanic *et al*, 1991; Koch *et al*, 1992; Koch and Franke, 1994), are the characteristic glycoproteins of these structures. Their prominent cytoplasmic plaques contain the common desmosomal plaque proteins, desmoplakin I and plakoglobin (Cowin *et al*, 1986; Franke *et al*, 1989; Franke *et al*, 1992). In the desmosomes of stratified cutaneous epithelia, further cytoplasmic plaque proteins have been identified, including desmoplakin II, a shorter splice variant of desmoplakin, and desmocalmin (Cowin *et al*, 1984; Cowin *et al*, 1985; Franke *et al*, 1992, Tsukita *et al*, 1992).

Recently, another major desmosomal plaque protein called plakophilin 1 has been identified in certain stratified (e.g., cutaneous) and complex epithelia; this protein was formerly reported as "band 6" protein of isolated bovine muzzle desmosomes, a positively charged polypeptide (molecular weight, 80,496) characteristically binding CKs *in vitro* (Kapprell *et al*, 1988; Heid *et al*, 1994; Schmidt *et al*, 1994). Bovine and human cDNA clones were obtained and the determination of the complete amino acid sequences of bovine and human plakophilin 1 indicated that plakophilin 1 is a member of the plakoglobin/armadillo protein family (Peifer and Wieschaus, 1990; Hatzfeld *et al*, 1994; Heid *et al*, 1994; Schmidt *et al*, 1994).

The skin presents a particularly complex variety of epithelia, comprising the interfollicular epidermis, the epithelia of eccrine sweat glands and sebaceous glands, the outer root sheath (ORS) and inner root sheath (IRS) epithelia of hair follicles, the hair shaft itself, and special neuroendocrine cells ("Merkel cells"). These

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Reprint requests to: Ingrid Moll, M.D., University of Heidelberg, Mannheim Medical School, Department of Dermatology, P.O. Box 23, 68135 Mannheim, Germany.

Abbreviations: CK, cytokeratin; BCC, basal cell carcinoma; SCC, squamous cell carcinoma.

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various epithelial cell types are known to express their specific CK genes. Thus, the keratinocytes of the epidermal basal cell layer express the CKs 5 and 14, the suprabasal prickle cells contain CKs 1, 10 and CK 2e (in parts), whereas the trichocytes of the hair follicle express at least eight major hair-type CKs (Moll *et al*, 1982a; Heid *et al*, 1986). Somewhat corresponding, recent mRNA and gene analyses have also shown that desmosomal cadherins are formed in the skin in relation to differentiation. Dsg 1 is predominantly found in upper prickle cells, whereas Dsg 2 is found in basal cells, and Dsg 3 is mainly found in the suprabasal compartment of interfollicular epidermis (Koch *et al*, 1992; Arnemann *et al*, 1993; Amagai *et al*, 1994; Schäfer *et al*, 1996). The genes for Dscs are also differentially expressed, Dsc 2 and 3 being found in desmosomes throughout the interfollicular epidermis, whereas Dsc 1 is confined to the suprabasal cell layers (Mechanic *et al*, 1991; Collins *et al*, 1991; Theis *et al*, 1993; King *et al*, 1993, 1995; Nuber *et al*, 1995; North *et al*, 1996; Nuber *et al*, 1996). This differential pattern of desmosomal proteins and glycoproteins may be related to differences of desmosomal morphology and complexity in these diverse epithelia (e.g., Lever and Schaumburg-Lever, 1975; Kocher *et al*, 1981; Tachikawa *et al*, 1984; Luzi *et al*, 1987). In order to gain more detailed insight into the specificity of the distribution of plakophilin 1 in desmosomes of normal and tumorigenic cutaneous tissues and its possible functions, we investigated the distribution of this member of the plakoglobin/armadillo protein family in samples of such tissues, including fetal skin and skin tumors.

MATERIALS AND METHODS

Immunohistochemistry Tissue samples of human skin and tumors obtained during routine surgery were immediately snap-frozen in isopentane pre-cooled with liquid nitrogen to about −130°C. Normal tissues (obtained from sites at least 2 cm distant from a lesion) from various body areas (scalp, trunk, axilla, sole, lip) were examined, as were four samples of psoriatic lesions (buttocks) and four samples of fetal skin (scalp, 14 and 21 weeks; trunk, 15 weeks; sole, 9 weeks) obtained after iatrogenic abortions performed for medical and nonmedical reasons (for estimation of the gestational age, see Streeter, 1920).

In addition, basal cell carcinomas (BCC; 21 cases) of various histologic types, cases of Bowen's disease (five cases), and squamous cell carcinomas (SCC; seven cases) were investigated. Keratinocyte sheets were cultured from adult body and sole skin according to routine methods (e.g., Rheinwald and Green, 1975; Gallico *et al*, 1984) to form monolayers and multilayered sheets, which were snap-frozen in pre-cooled isopentane immediately after dispase digestion.

Cryostat sections (~5 μm thick) were prepared from the frozen tissue blocks, and immunofluorescence and immunoperoxidase microscopy were performed essentially as described elsewhere (Franke and Moll, 1987; Moll and Moll, 1991; Heid *et al*, 1994). In most experiments, primary antibodies were three of the specific monoclonal antibodies directed against plakophilin 1 (PP1-5C2, PP1-2D6, PP1-9E7; for antibody characterization, see Heid *et al*, 1994; Schmidt *et al*, 1994); a monoclonal plakoglobin antibody (PG 5.1.7.2), and two monoclonal desmoplakin antibodies (DP 1 and 2–215, DP 1–217; all available from Progen Biotechnics, Heidelberg, Germany; Cowin *et al*, 1985). In addition, the monoclonal CK antibodies, K_G 8.60 against CKs 1/10/11, available from Biomakor (Rehovot, Israel), E3 (against CK 17; Progen Biotechnics), and VIM-9 (against vimentin: available from Viramed, Martinsried, Germany) were applied. For the plakophilin 1 antibodies, in some cases a pre-treatment of the sections using 0.1% Triton X-100 in phosphate-buffered saline for 10 min was performed immediately prior to application of the primary antibody. Controls were performed using antibody K_S 18.174 against CK 18 (Progen Biotechnics), which is absent from epidermal keratinocytes (for its distribution in Merkel cells, see Moll *et al*, 1984a) or 0.1 M/phosphate-buffered saline instead of a specific primary antibody. For immunofluorescence microscopy, mainly Texas red–labeled rabbit antibodies against mouse immunoglobulins (Dianova, Hamburg, Germany) or dichlorotriacinylaminofluorescin-labeled rabbit antibodies against mouse immunoglobulins (Dianova) were used as secondary antibodies. Indirect immunoperoxidase staining was performed using peroxidase-coupled goat antibodies against mouse immunoglobulins (Dako, Hamburg, Germany) as the secondary antibodies. In some experiments, the avidin-biotin-peroxidase complex method (ABC Kit; Vector, Burlingame, CA) was used. 3,3′-Diaminobenzidine and H_2O_2 were applied for visualization (see Franke and Moll, 1987).

Gel Electrophoresis and Immunoblotting Desmosomal protein fractions from adult epidermis were prepared and analyzed using non-equilibrium–pH-gradient gel electrophoresis (first dimension) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (second dimension) as described previously (for details see Heid *et al*, 1994). The separated proteins were blotted to nitrocellulose, and antibody PP1-5C2 was used for immunodetection with peroxidase-coupled anti-mouse antibodies and the chemiluminescence system (ECL; Amersham, Braunschweig, Germany; Heid *et al*, 1994).

RESULTS

Presence of Desmosomal Plakophilin 1 in Human Adult Skin In the various epithelia of human skin, immunohistochemistry was applied to localize plakophilin 1. In interfollicular epidermis, plakophilin 1 was detected in desmosomes from the basal to the granular layer, staining the basal cell layer weakly and the upper prickle cells more intensely **(Fig 1*a*)**. This indicates that plakophilin 1 is already present in keratinocytes at an earlier state of differentiation than, for example, CKs 1 and 10, which are restricted to suprabasal cells (Weiss *et al*, 1984; for review see Fuchs, 1995). In sufficiently thin sections, and especially using conventional immunofluorescence, the reaction sites are frequently resolved into the individual punctate pattern typical of desmosomal staining, even on cells of the basal layer that contain lower numbers of desmosomes **(Fig 1*a*)**. In immunoperoxidase staining of frozen tissues, however, this dotted desmosomal pattern is often not resolved, and a more lining pattern is observed due to technical reasons **(Fig 1*b*)**. Moreover in the basal cell layer the reaction sites are restricted to the lateral and upper cell surface whereas their basal cell surface (corresponding to the basal lamina) appears to be entirely unreactive **(Fig 1*a,b*)**. There is no detectable reaction in the stratum corneum **(Fig 1*a,b*)**. The labeling patterns using the three plakophilin 1 antibodies are essentially identical, with a somewhat lower staining intensity when the PP1-2D6 antibody is used. In comparison, the antibody directed against plakoglobin, another typical desmosomal plaque protein, also intensely decorated the upper and lateral surface of basal cells but not their basal surface. The lower suprabasal cells are prominently demarcated, whereas upper prickle and granular cells are only weakly stained, and the stratum corneum remains unstained **(Fig 1*c*)**.

Interestingly, the distribution of plakophilin 1 in the various hair follicle epithelia is rather heterogenous. The infundibular epithelia exhibit a desmosomal distribution pattern very similar to that observed in the interfollicular epidermis (compare **Fig 1 *a-c***). The ORS is mainly decorated in the form of the desmosomes of its suprabasal (inner) cell layers, where the staining is more intense in its upper two-thirds than in the lower, suprabulbar, third. Moreover, in the upper part of the ORS, the innermost cells are unequivocally stained **(Fig 1*d*)**; this is in contrast to such cells in the lower part **(Fig 1*e*)**. The cuboid basal cells and (often) parabasal cells of the bulge area **(Fig 1*d*)**, the columnar basal cells in the central third of the ORS **(Fig 1*e*)**, as well as ORS cells around the bulb, were not decorated by the plakophilin 1 antibodies applied. A weak reaction at their upper cell surface bordering suprabasal cells, however, is often detectable **(Fig 1*d,e*)**. The undifferentiated matrix cells below the line of Auber are also unstained, but during their further differentiation toward trichocytes of the hair shaft, these cells and the lower parts of the hair shaft became stained at their cell border (not shown). Moreover, to compare the plakophilin 1-negative basal keratinocytes of ORS, a "general" desmosomal marker, namely desmoplakin antibodies, was applied. These antibodies clearly demonstrated reactivity in basal cells of ORS also, arguing for the presence of desmosomal plaques **(Fig 1*f*)**. The decoration of the IRS by plakophilin 1 antibodies is also interesting, being reactive within the bulb from its very early beginning but clearly switching to negative above the bulb **(Fig 1*d,e*)**.

In eccrine glands, the desmosomal immunostaining of plakophilin 1 is heterogeneous. In the dermal duct portion, the ductal lining cells are stained at their cell borders **(Fig 2*b*)**. Their lateral and upper cell surfaces are reactive, whereas their lumen-bordering

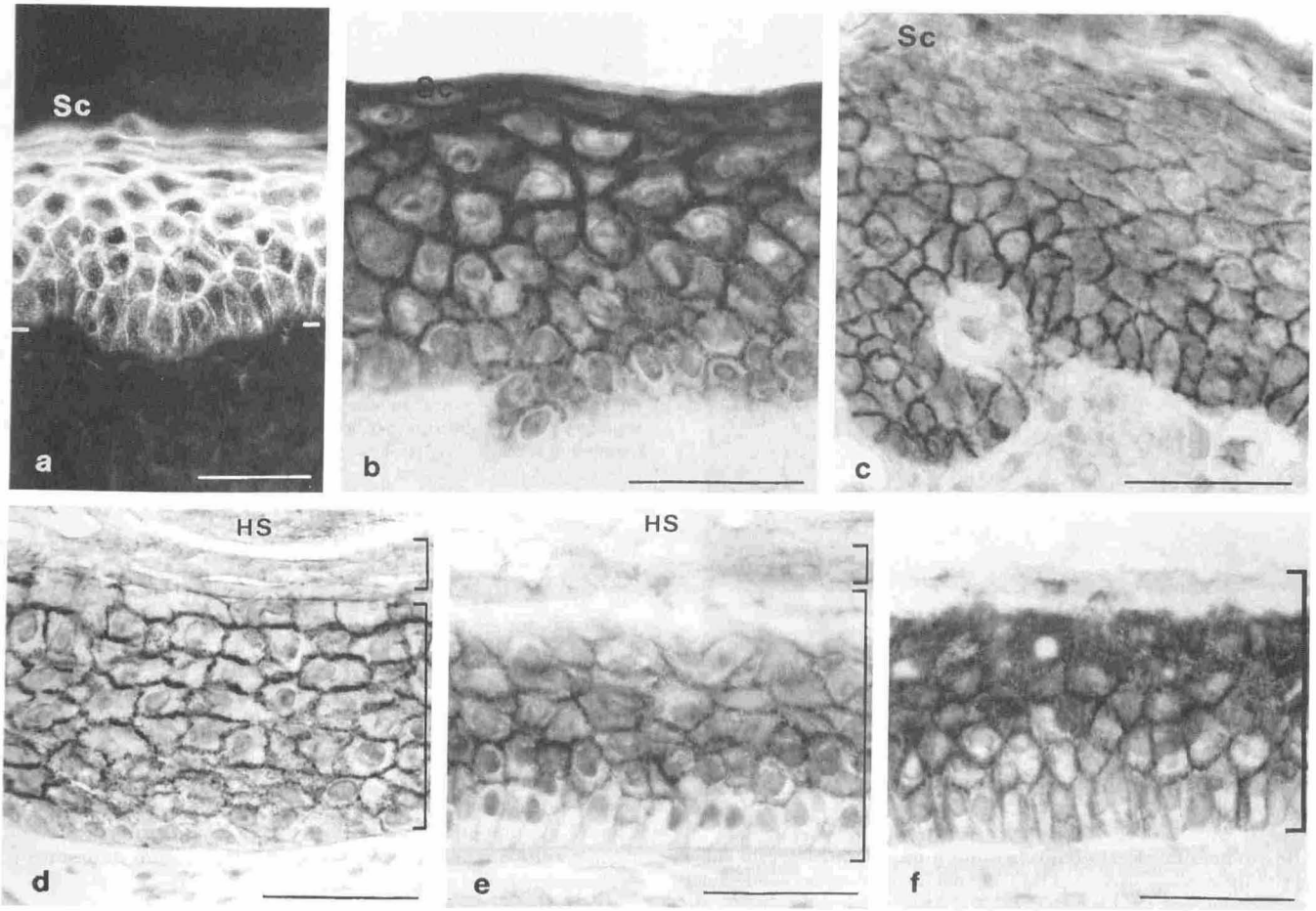

Figure 1. ***(a-d)*** **Immunofluorescence** ***(a)*** **and immunoperoxidase** ***(b-f)*** **microscopy of adult skin using antibody PP1-9E7 selective for plakophilin 1** ***(a,b,d,e)*****, antibody PG 5.1.72 against plakoglobin** ***(c)*** **and antibody DP 2.17 against desmoplakin I and II (f).** *(a)* The interfollicular epidermis is decorated weakly in basal cells and strongly in suprabasal cell layers; the stratum corneum (Sc) is unstained. Note the dotted staining pattern most prominently in basal cells. The basement membrane is marked at both sides. *(b,c)* The basal cells are weakly decorated, but the suprabasal ones prominently *(b)*, whereas the plakoglobin antibody marks the basal and lower suprabasal keratinocytes intensely but the upper ones only weakly. *(d)* The ORS at the level of bulge is stained in its suprabasal cells including its innermost, but not in basal ones; IRS cells are unstained. *(e, f)* The central cells of the lower third of the ORS are labeled intensely, its innermost cells only weakly. The tall, thin basal cells of this lower ORS are unstained. In contrast, the latter cells are unequivocally decorated by the desmoplakin antibodies *(f)*. *Large bracket*, ORS; *small bracket*, IRS; HS, hair shaft. *Scale bars*, 50 μm

surface is observed to be unmarked. Also the ductal basal cells and the cells of acini remain without desmosomal decoration **(Fig 2*a,b*)**. These data are summarized in **Table I**.

Immunolocalization of Plakophilin 1 in Fetal Skin Plakophilin is detectable within desmosomes of fetal epidermis obtained at about week 9 of gestation, being most prominently localized on the upper surface of basal cells and intermediate cells, but the basal and lateral surface of basal keratinocytes are unreactive **(Fig 3*a*)**. Peridermal cells remained essentially unstained **(Fig 3*a*)**. Some weeks later, at fetal week 15, the two to three intermediate cell layers are decorated but somewhat heterogenously **(Fig 3*b*)**. At the lateral cell surface of basal cells there is only a weak immunoreaction detectable when the three plakophilin 1 antibodies are used **(Fig 3*b*)**.

Biochemical Detection of Plakophilin 1 in Human Skin In cytoskeletal preparations of human sole epidermis after two-dimensional gel electrophoresis, the major type I CKs (nos. 9, 10, 11, 14, 15), and type II CKs (nos. 1, 2e, 5), are clearly observed, whereas the plakophilin 1 is not seen on these Coomassie stained gels **(Fig 4*a*)**. Using the monoclonal antibody PP1-5C2 in western immunoblotting, plakophilin 1 could be detected at its typical position **(Fig 4*b*)**.

Plakophilin 1 Is Detectable in Psoriatic Epidermis Immunostainings of psoriatic lesions reveal a desmosomal heterogenous weak to strong staining pattern within prickle cell layers. The one to three basal type cell layers are only weakly stained, and the stratum corneum is unreactive **(Fig 5)**.

Heterogenous Immunolocalization of Plakophilin 1 in BCCs Having studied the heterogeneous desmosomal immunolocalization pattern of plakophilin 1 in the various epithelia of the skin, we extended our investigation to epithelial skin tumors. Morphologically, BCCs encompass a broad spectrum of undifferentiated solid types as well as tumors exhibiting differentiation toward several adnexal structures. In addition, morphaea-like types demonstrating only small groups of epithelial strands embedded in masses of fibrous stroma exist. Consequently, the epithelial cell-cell adhesions of BCCs represent an interesting field of investigation. In solid, nodular BCCs, the desmosomal distribution of plakophilin 1 is detectable throughout the tumor, although the staining intensity is much weaker in general than in the overlying epidermis **(Fig 6*a*)**. The switch to very weak immunostaining is evident in solid tumor nodules growing close to the basal epidermis **(Fig 6*a*)**. In contrast, the application of desmoplakin antibodies to consecutive sections results in an intense staining of the BCC nodules, which was similar

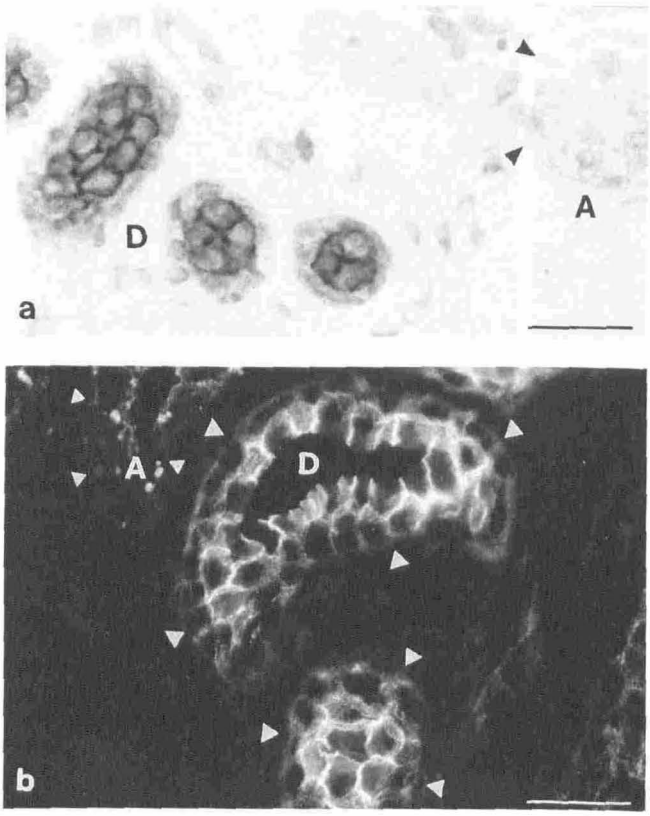

Figure 2. Immunoperoxidase *(a)* and immunofluorescence *(b)* staining of eccrine sweat glands in adult human skin. The cells lining ducts (D) are intensely decorated *(a,b)*, but not their lumen-bordering inner cell surface and not the basal cells (b, ➤). Acini (A, ➤), in contrast are unstained *(a, b)*. *Scale bars*, 50 μm.

to the staining of the epidermis **(Fig 6*b*)**. In the solid tumor areas, plakophilin 1 antibodies frequently elicit a very heterogeneous and patchy staining of desmosomes, exhibiting small foci of immunostained cells **(Fig 6*c*)**. In adenoid types of BCC, the distribution pattern of plakophilin 1 is also very heterogeneous **(Fig 6*d*)**. In contrast, in morphaea-like BCCs, plakophilin 1 immunostaining is extremely restricted, being mainly confined to the desmosomes of central cells of some epithelial cords **(Fig 6*e*)**. Interestingly, a comparable localization of plakophilin 1 and the suprabasal-type CK 1/10 is frequently demonstrable in consecutive sections using antibodies against plakophilin 1 **(Fig 6*f*)** and CK 1/10 **(Fig 6*g*)**, but the distribution of desmosomal plakophilin 1 extends over somewhat larger areas. Thus, the distribution pattern of plakophilin 1 and CKs 1/10 in BCCs corresponds to that found in normal human epidermis **(Fig 6*f,g*)**.

Table I. Distribution of Plakophilin 1 in Human Skin

Epidermis	Basal (+)	S. spin. ++	S. corn. −
Hair follicle			
Infundibulum	Basal (+)	Suprabasal ++	S. corn. −
ORS-upper two-third	Basal −	Suprabasal ++	Innermost +
ORS-lower third	Basal −	Suprabasal ++	Innermost (+)
Bulb	Basal −	Medium +	uppermost +
IRS-upper two-third	Outer −	Inner −	
IRS-lower third	Outer +	Inner −	
Eccrine gland			
Acinus	−	−	
Dermal duct	Basal −	Suprabasal ++	

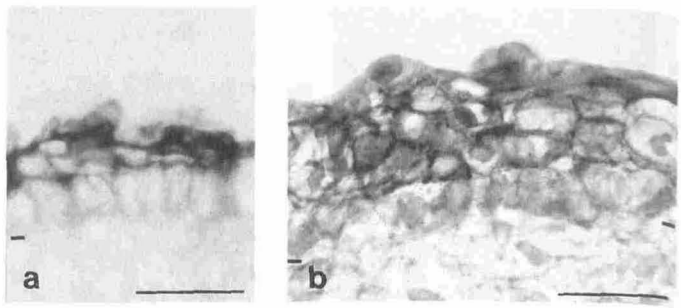

Figure 3. Immunoperoxidase microscopy of fetal skin (palmar, 9 wk *(a)* and body, 15 wk *(b)* using the antibody, PP1-9E7. *(a)* The intermediate cells are positive; the basal and peridermal cells are unstained. *(b)* Again the two to three intermediate cells are marked, the basal cells are weakly decorated, but the peridermal cells are unstained. The basement membrane is marked by strokes. *Scale bars*, 50 μm.

Immunolocalization of Plakophilin 1 in SCCs and Bowen's Disease SCCs comprise strands of epidermal cells that proliferate into the dermis and are composed of atypical prickle cells. In the well-differentiated squamous areas, as in epidermis, there is very intense desmosomal staining for plakophilin 1 **(Fig 7*a*)**, whereas there is little or no staining in the more invasive, undifferentiated areas that often preferentially express simple epithelial-type CKs (e.g., nos. 18, 19; **Fig 7*b*)**. In particular, the most invasively growing areas deep within the dermis are very weakly or entirely unstained **(Fig 7*b*)**. Interestingly, the unstained areas of one case are decorated by vimentin antibodies while the other cases are unstained by vimentin antibodies. As in BCCs, it proved possible to demonstrate a correlation between the distribution of desmosomal plakophilin 1 and CKs 1/10 (data not shown).

In contrast, in Bowen's disease, an *in situ* carcinoma of the epidermis, the majority of tumor cells are intensely immunostained by the plakophilin 1 antibodies at their cell borders, but some minor ones are weakly immunostained **(Fig 7*c*)**.

Presence of Plakophilin 1 in Keratinocyte Sheets *in Vitro* As it was possible to identify desmosomal plakophilin 1 in keratinocytes of the epidermis and in the well-differentiated areas of epithelial skin tumors in general, it was also of interest to investigate whether this protein occurs in keratinocyte cultures. Keratinocytes of sole and body epidermis were grown in culture as monolayers and as stratified sheets such as those used for transplantation (Limat *et al*, 1996). In stratified sheets (three to five layers) plakophilin 1 is found to be present in the desmosomes of suprabasal cell layers but not in those of basal cells **(Fig 8)**.

DISCUSSION

In the present study it proved possible to demonstrate the presence and distribution of plakophilin 1 at sites of desmosomes in the interfollicular epidermis and skin adnexa, as well as in various epithelial skin tumors and keratinocyte sheets *in vitro*. The typical dotted staining patterns preferentially seen in very thin samples after conventional immunofluorescence staining are in line with the notion that, along cell borders, the protein is localized in desmosomes (see also Heid *et al*, 1994). Plakophilin 1 is a basic protein (molecular weight, 80,496; IEP 9.34) and, according to its amino acid sequence, a member of the plakoglobin/β-catenin/armadillo protein-gene family (Kapprell *et al*, 1988; Hatzfeld *et al*, 1994; Heid *et al*, 1994; Schmidt *et al*, 1994). Desmosomal staining of plakophilin 1 is more clearly detectable especially in the higher differentiated suprabasal cell layers of the epidermis, the ORS of hair follicles, the dermal ducts, and, to a weaker extent, in basal keratinocytes of epidermis. In accordance with these observations, plakophilin 1 is detected in and around areas of BCCs and SCCs composed of prickle-like cells, which are also positive for CKs 1/10 and are principally located at the center of tumor nodules. Plakophilin 1 is

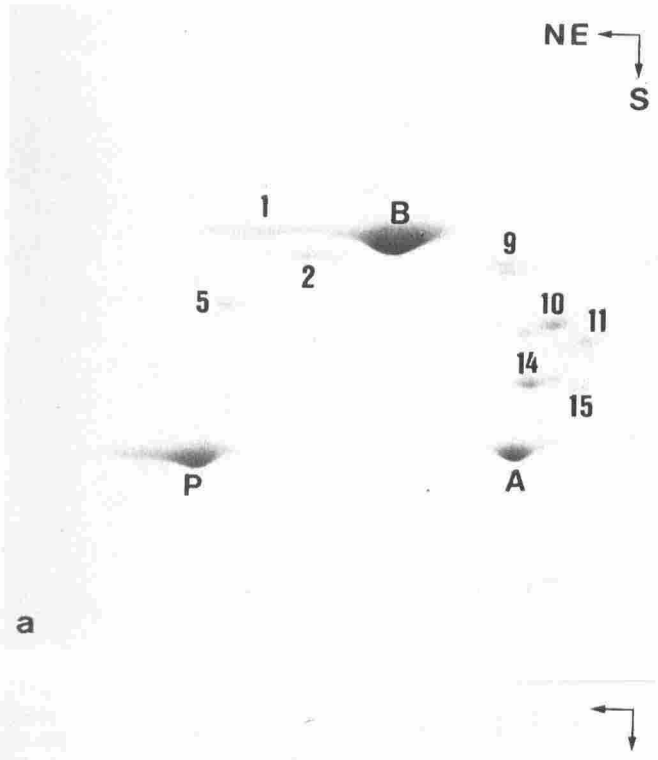

Figure 4. *(a,b)* Detection of plakophilin 1 in human sole epidermis. A cytoskeletal preparation was separated by non-equilibrium–pH-gradient gel electrophoresis (NE) in the first dimension (→) and by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (S) in the second dimension (↑). Rabbit muscle actin (A), bovine serum albumin (B), and rabbit muscle-3-phosphoglycerokinase (P) were co-electrophoresed as reference proteins. *(a)* Coomassie blue staining showing the major human cytokeratins (CK); numbers are according to Moll *et al* (1982a). *(b)* Immunoblot using monoclonal antibody PP1-5C2. Note the specific reaction at about M_r 75,000, isoelectric pH range 8.5 to 9, as seen by chemiluminescence (ECL) detection.

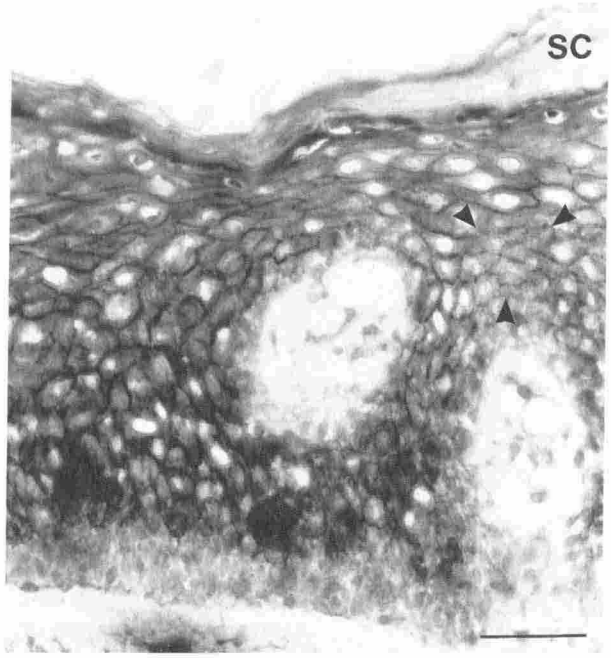

Figure 5. Immunoperoxidase microscopy of psoriatic epidermis using the plakophilin 1 antibody PP1-9E7. The lesions are stained somewhat heterogeneously; a weakly stained area is marked (➤) and some basal layers remain unstained. SC, Stratum corneum. *Scale bar*, 50 μm.

hardly expressed in desmosomes of less differentiated, invasive tumor areas that also might contain lower amounts of desmosomes (Luzi *et al*, 1987). In accordance, the distribution pattern of plakophilin 1 in tissues emerges as being correlated to that of CKs 1/10 both in normal and psoriatic epidermis as well as in fetal epidermis (Moll *et al*, 1982b). In eccrine dermal ducts, plakophilin 1 is found to be present in the innermost, suprabasal layer but is absent from those of basal ductal cells and acini. Again, main differentiation products of these plakophilin 1 positive cells are CKs 1/10, along with CK 19 in these cases (Moll *et al*, 1982a, 1982b; Moll and Moll, 1991). The distribution of plakophilin 1 and CKs 1/10 positive cells, however, is not identical, because plakophilin 1 is present, although weakly, in the desmosomes of the basal layer and of normal and psoriatic epidermis, whereas CKs 1/10 are absent (Weiss *et al*, 1984; Fuchs, 1995). The same is true in tumor nodules, where cells bearing plakophilin 1 in desmosomes are more widely distributed than CKs 1/10, which are confined to the tumor centers of BCCs and SCCs with the highest differentiation. On the other hand, plakophilin 1 and CKs 1/10 positive cells may be completely dissociated, e.g., in the ORS. At this site, CK 17 is the important differentiation marker of the suprabasal keratinocytes positive for plakophilin 1 (Moll, 1995). Thus, plakophilin 1 is predominant in desmosomes of cells in distinctly differentiated cutaneous stratified epithelia, adnexa, and neoplasms but is sparsely distributed or even absent in undifferentiated areas of epithelia, e.g., basal epidermal keratinocytes (sparse), basal cells of ORS, and hair matrix cells (absent).

In contrast, desmoplakin I and plakoglobin are probably present in all desmosomes occurring in the various cutaneous epithelia (Cowin *et al*, 1984, 1986; Franke *et al*, 1992; Schmidt *et al*, 1994). Indeed, the presence of these proteins is clearly demonstrated in the upper and lateral surface of basal cells of the interfollicular epidermis and of the ORS. This reactivity may also depend on the presence of plakoglobin in adherens junctions (Cowin *et al*, 1986).

In human hair follicles, the desmosomes of cells of the basal layer of the ORS are unstained by the plakophilin 1 antibodies used. Within its zone of broadening and of bulge, two or three additional parabasal cell layers remain undecorated. These cells exhibit many (broadening area; Moll, 1995) or few (bulge area; Cotsarelis *et al*, 1990) mitotic active keratinocytes, with proliferation apparently being independent of the presence of desmosomal plakophilin 1 at these sites. Moreover, in hair follicles, plakophilin 1 antibodies react variably within deep and upper parts, with sudden switches being noticeable. Whether this represents regular changes of

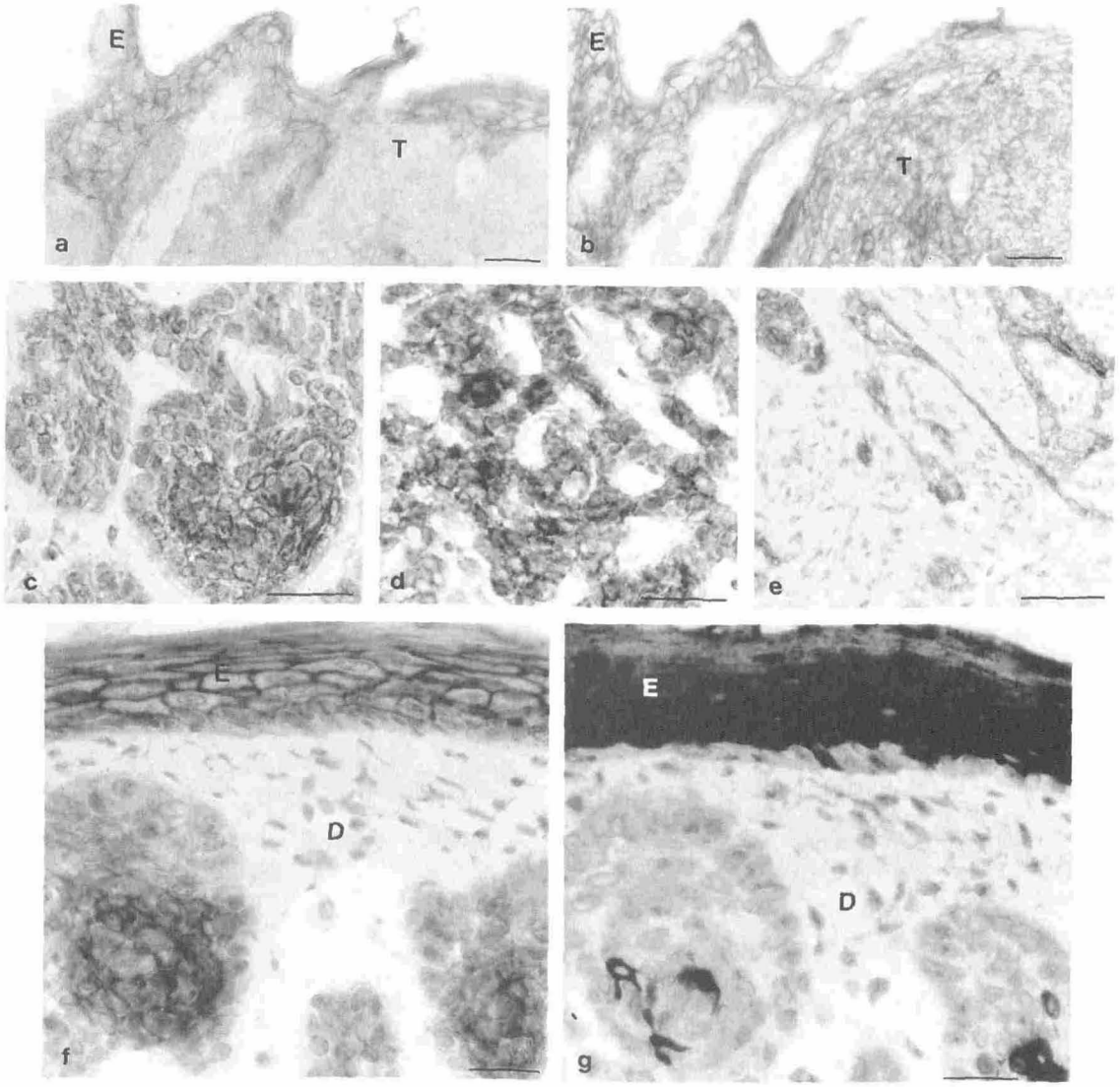

Figure 6. Immunoperoxidase microscopy of BCCs using antibodies against plakophilin 1 [PP1-9E7 *(c,d,e,f)*; PP1-2D6 *(a)*], desmoplakins [DP 1 and 2–215 *(b)*], and CKs 1/10 [K_G8.60 *(g)*]. *(a,b)* The epidermis (E) overlying a superficial BCC is brightly decorated whereas the tumors (T) itself stains weakly and heterogeneously. Note the immediate switch of reaction at the border of the tumor. A consecutive section *(b)* showing for comparison a desmoplakin staining. Here in contrast the epidermis (E) and the tumor (T) are identically and homogenously decorated. *(c,d) Solid (c) and adenoid (d)* BCCs are very heterogenously stained showing decorated tumor nodules *(c)* and disseminated staining patterns of adenoid strains *(d)*. **(e)** In sclerodermiform BCC, antibody PP1-9E7 stains only rare cells mostly within tumor strains. *(f,g)* Consecutive sections of a solid BCC and the overlying epidermis. Antibody PP1-9E7 stains the suprabasal keratinocytes and largely the centers of tumor nodules, whereas the periphery remains unstained. The distribution of CK 1/10-positive cells *(g)* is restricted to the middle of the plakophilin-positive areas *(f)*. E, epidermis; D, dermis. *Scale bars*, 50 μm.

plakophilin 1 in desmosomal composition during hair cycles remains to be clarified. Interestingly, in the central (suprabasal) keratinocytes of the bulge, cells that are generally thought to be low differentiated (Akiyama *et al*, 1995), we were able to detect plakophilin 1 at the cell borders, which would suggest that these cells are probably, in some respect, at an advanced stage of differentiation (see also Cotsarelis *et al*, 1990).

The possible functions of plakophilin 1 as a component of desmosomes must, as yet, remain a matter for speculation. This protein appears not to be obligatory for the formation and functioning of all desmosomes, as desmosomes lacking plakophilin 1 seem to exist, e.g., at the lateral surfaces of basal cells of ORS and in sites of BCC and SCC. On the other hand, one might argue that there may be problems with respect to recognition of the respective epitope of the used antibodies, these apparently stemming from antigen-masking phenomena that are probably correlated with the stages of differentiation of the tissue under investigation. We used three different plakophilin 1 antibodies, however, resulting in nearly identical desmosomal distribution patterns, which argue against such detection problems and for being representative of desmosomal distribution of plakophilin 1.

The fact that plakophilin 1 is a highly conserved molecule during evolution (for homologues, see Peifer and Wieschaus, 1993; Heid *et al*, 1994; Peifer *et al*, 1994) should suggest its considerable importance for differentiation of keratinocytes in epithelia. As it has been shown that, *in vitro*, plakophilin 1 binds CKs in binding assays (Heid *et al*, 1994; Schmidt *et al*, 1994). It may function as a structural linker for intermediate filaments, on the one hand, and for the desmosomal cadherins, on the other (Hülsken *et al*, 1994). In addition, as plakophilin 1 is a member of the armadillo protein gene family it may, in addition, act as an important signal-transducing molecule similar to the armadillo protein in *Drosophila* (Peifer and Wieschaus, 1993; Peifer *et al*, 1994).

Moreover it would thus appear that the less differentiated, more invasively growing areas of BCC and SCC contain less desmosomal plakophilin 1 than the higher differentiated areas of these tumors.

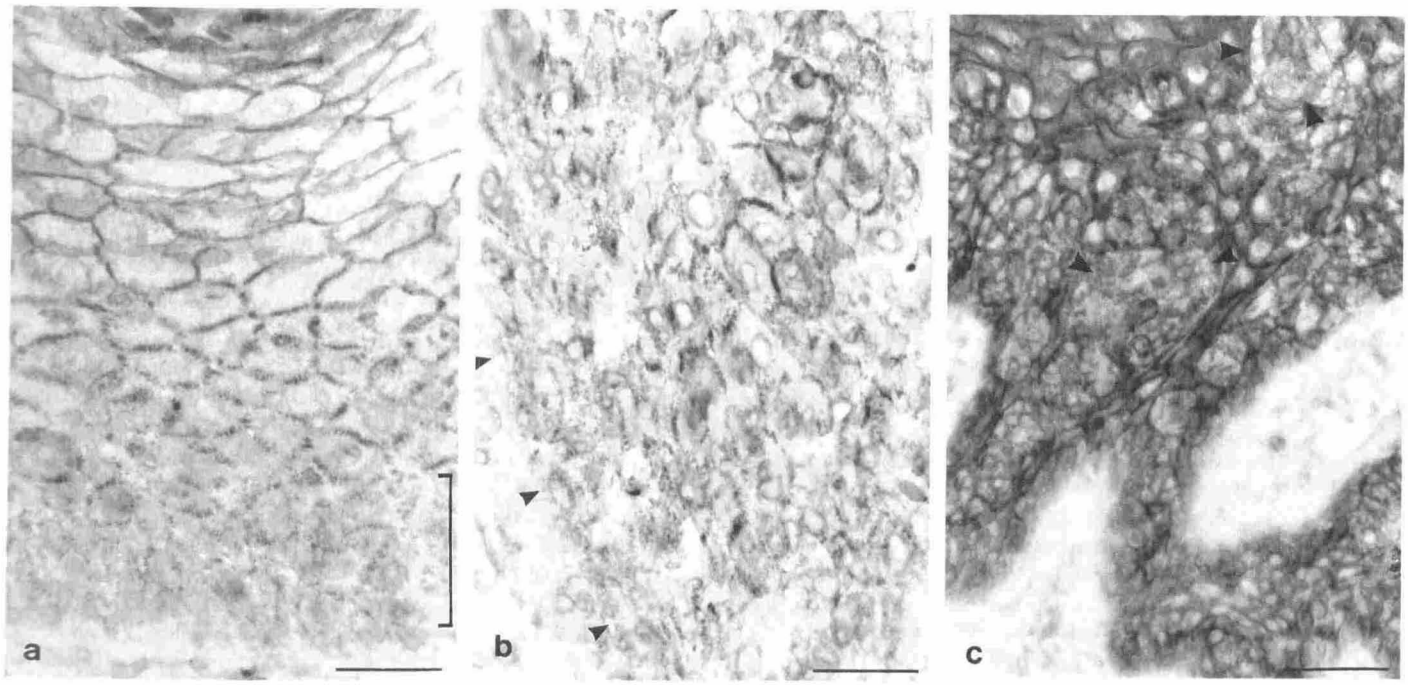

Figure 7. (a-c). Immunoperoxidase microscopy using antibody PP1-9E7 against plakophilin in SCCs *(a,b)* and Bowen's disease *(c).* *(a,b)* In well-differentiated, cornifying areas of the SCCs plakophilin 1 is prominent (*a*, upper part); in less differentiated areas, *(a, bracket; b)* its distribution diminishes until almost negative. ➤ mark the border of tumor (*b*). *(c)* In Bowen's disease, the immunostaining is heterogeneous, with small cells often being weakly stained (➤). *Scale bar*, 50 μm.

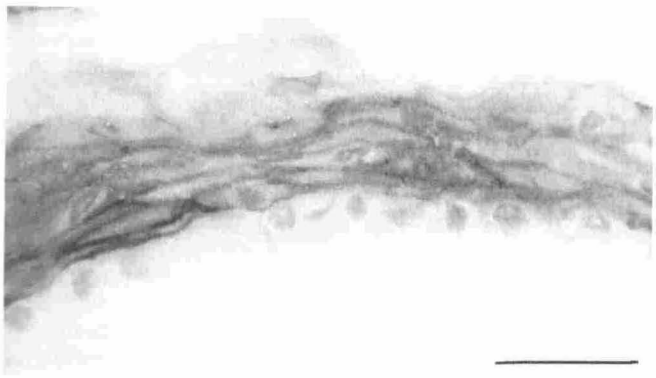

Figure 8. Immunoperoxidase microscopy *(a)* of keratinocyte sheet (developed from sole epidermis) using a plakophilin-1 antibody PP1-9E7. The suprabasal, flat keratinocytes are intensely stained, whereas the basal cells are undecorated. *Scale bar*, 50 μm.

As plakophilin 1 protein is observed in desmosomes of cells at higher stages of differentiation, its absence or presence may be more directly correlated with the cell-cell contacts and, therefore, with the biologic behavior of malignant tumors than various cytoplasmic differentation markers, e.g., CKs 1/10 (Winter *et al*, 1983; Moll *et al*, 1984b). In this context the study of lymph node metastases of SCCs should be of interest. Furthermore, the distribution of desmosomal plakophilin 1 in bullous diseases of skin, especially in genetic disorders such as Hailey-Hailey disease and Darier disease, should also be of interest, as plakophilin 1 as a constituent in desmosomes could possibly be involved in mechanisms of acantholysis that might account for such genetic disorders.

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